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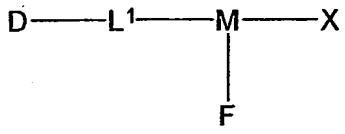
Claims

1. A compound selected from an acridone and a quinacridone dye containing at least one target bonding group selected from a carboxylic acid thioester group or a group suitable for covalent reaction with a thioester, wherein said compound optionally includes an affinity tag covalently bound thereto.

2. A compound according to claim 1 having the formula (I):



(I)

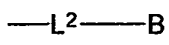
wherein:

D is a fluorescent dye selected from an acridone and a quinacridone dye;

F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group;

M is a group adapted for attaching to F;

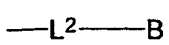
X is selected from hydrogen or the group:



wherein B is an affinity tag; and

L^1 and L^2 each independently comprise a group containing from 1 – 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from –NR'–, –O–, –CH=CH–, –CO–NH– and phenylenyl groups, where R' is selected from hydrogen and C_1 – C_4 alkyl.

3. A compound according to claim 2 wherein X is the group:



wherein B and L^2 are hereinbefore defined.

4. A compound according to claim 2 or claim 3 wherein each of L^1 and L^2 contains from 2 to 30 atoms.

5. A compound according to claim 2 or claim 3 wherein L^1 and L^2 are independently selected from the group:

$$-\{(CHR')_p-Q-(CHR')_r\}_s-$$

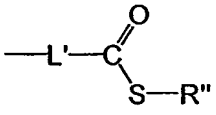
where Q is selected from: –CHR'–, –NR'–, –O–, –CH=CH–, –Ar– and –CO–NH–; R' is hydrogen or C_1 – C_4 alkyl, p is 0 – 5, r is 1 – 5 and s is 1 or 2.

6. A compound according to claim 5 wherein Q is selected from –CHR'–, –O– and –CO–NH–, where R' is hereinbefore defined.

7. A compound according to any of claims 1 to 6 wherein said affinity tag is selected from biotin and desthiobiotin.

8. A compound according to any of claims 1 to 6 wherein said affinity tag is selected from his-tag, iminodiacetic acid and nitrilotriacetic acid.

9. A compound according to any of claims 1 to 8 wherein the target bonding group F is a carboxylic acid thioester of formula:

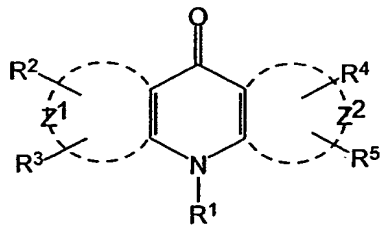


wherein L' is a bond or is a group containing from 1 – 30 linked atoms selected from carbon atoms and optionally one or more groups selected from –NH–, –O– and –CO–NH–; and R" is C_1 – C_4 alkyl, C_6 – C_{10} aryl, or C_7 – C_{15} aralkyl, which may be optionally substituted with sulphonate; or is the group $-(CH_2)_2-CONH_2$.

10. A compound according to any of claims 1 to 8 wherein the target bonding group F is a 1,2-aminothiol group of formula:

wherein L' is hereinbefore defined.

11. A compound according to any of claims 1 to 10 wherein the compound is an acridone dye having the formula (II):



(II)

wherein:

groups R^2 and R^3 are attached to the Z^1 ring structure and groups R^4 and R^5 are attached to the Z^2 ring structure;

Z^1 and Z^2 independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

at least one of groups R^1, R^2, R^3, R^4 and R^5 is a group W having the formula:

where F, M, X and L^1 are hereinbefore defined;

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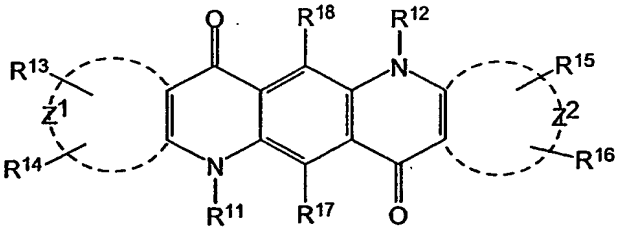
when any of said groups R^1, R^2, R^3, R^4 and R^5 is not said group W, said remaining groups R^2, R^3, R^4 and R^5 are independently selected from hydrogen, halogen, amide, cyano, mono- or di-$C_1 - C_4$ alkyl-substituted amino, carbonyl, carboxyl, $C_1 - C_6$ alkoxy, acrylate, vinyl, styryl, aryl, heteroaryl, $C_1 - C_{20}$ alkyl, aralkyl, sulphonate, sulphonic acid, quaternary ammonium and the group $-(CH_2)_n-Y$ and,

when group R^1 is not said group W, it is selected from hydrogen, $C_1 - C_{20}$ alkyl, aralkyl and the group $-(CH_2)_n-Y$; and

Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl; and n is an integer from 1 to 6;

provided that at least one of groups R^1, R^2, R^3, R^4 and R^5 is a water solubilising group.

12. A compound according to any of claims 1 to 10 wherein the compound is a quinacridone dye having the formula (III):



(III)

wherein:

groups R^{13} and R^{14} are attached to the Z^1 ring structure and groups R^{15} and R^{16} are attached to the Z^2 ring structure;

Z^1 and Z^2 independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

at least one of groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17} and R^{18} is a group T having the formula:

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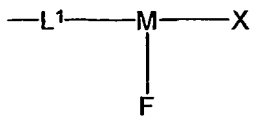

where F, M, X and L^1 are hereinbefore defined;
when any of said groups R^{13}, R^{14}, R^{15}, R^{16}, R^{17} and R^{18} is not said group T, said remaining groups R^{13}, R^{14}, R^{15}, R^{16}, R^{17} and R^{18} are independently selected from hydrogen, halogen, amide, cyano, mono- or di-$C_1 - C_4$ alkyl-substituted amino, carbonyl, carboxyl, $C_1 - C_6$ alkoxy, acrylate, vinyl, styryl, aryl, heteroaryl, $C_1 - C_{20}$ alkyl, aralkyl, sulphonate, sulphonic acid, quaternary ammonium and the group $-(CH_2)_n-Y$; and,
when either of groups R^{11} and R^{12} is not said group T, it is selected from hydrogen, $C_1 - C_{20}$ alkyl, aralkyl and the group $-(CH_2)_n-Y$;
Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl; and n is an integer from 1 to 6;
provided that at least one of groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17} and R^{18} is a water solubilising group.

13. A compound according to claim 11 or claim 12 wherein Z^1 and Z^2 are selected independently from the group consisting of phenyl, pyridinyl, naphthyl, quinolinyl and indolyl moieties.

14. A compound according to claim 11 or claim 12 wherein Z^1 and Z^2 are selected from phenyl and naphthyl moieties.

15. A method for labelling a protein of interest wherein said protein contains or is derivatised to contain an N-terminal cysteine, the method comprising:

i) adding to a liquid containing said protein a compound of formula (I):

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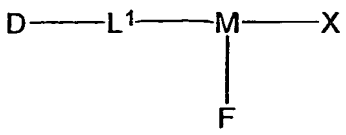

(I)

wherein:

D is a fluorescent dye selected from an acridone and a quinacridone dye;

F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group;

M is a group adapted for attaching to F;

X is selected from hydrogen or the group:

$$—L^2——B$$

where B is an affinity tag; and

L^1 and L^2 each independently comprise a group containing from 1 – 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from –NR'–, –O–, –CH=CH–, –CO–NH– and phenylenyl groups, where R' is selected from hydrogen and C_1 – C_4 alkyl; and

ii) incubating said compound with said protein under conditions suitable for labelling said protein.

16. A compound according to claim 15 wherein each of L^1 and L^2 contains from 2 to 30 atoms.

17. A method according to claim 15 wherein L^1 and L^2 are independently selected from the group:

$$–\{(CHR')_p–Q–(CHR')_r\}_s–$$

where Q is selected from: –CHR'–, –NR'–, –O–, –CH=CH–, –Ar– and –CO–NH–; R' is hydrogen or C_1 – C_4 alkyl, p is 0 – 5, r is 1 – 5 and s is 1 or 2.

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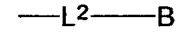
18. A method according to claim 17 wherein Q is selected from –CHR'–, –O– and –CO–NH–, where R' is hereinbefore defined.

19. A method according to any of claims 15 to 18 wherein X is the group:



wherein B and L^2 are hereinbefore defined, said method further comprising separating and/or purifying the dye-labelled protein of interest by affinity chromatography.

20. A method according to any of claims 15 to 19 wherein said protein of interest is selected from antibody, antigen, protein, peptide, microbial materials, cells and cell membranes.